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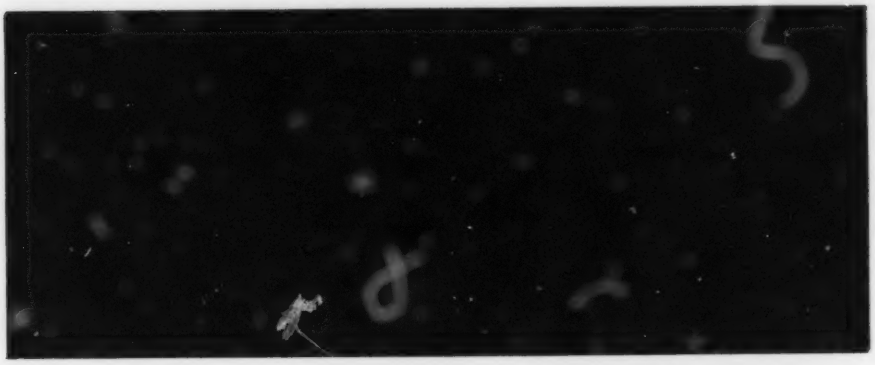

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Final Report

2.4-22

Immunopathogenesis of Bovine Herpesvirus-1 (BHV-1) Infection in Calves

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FINAL REPORT

IMMUNOPATHOGENESIS OF BOVINE HERPESVIRUS-1 (BHV-1) INFECTION IN CALVES

PROJECT NO. 2.4-22
Canada/Saskatchewan ERDA Agreement

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PROJECT NO. 2.4-22, FINAL REPORT, MARCH 1990

(a) EXECUTIVE SUMMARY

Abstract

Bovine herpesvirus-1 (BHV-1) causes several economically important diseases of cattle, including infectious bovine rhinotracheitis and death of neonatal calves. The reason for the fatal infection in neonates is poorly understood.

The objective of this research was to compare the pathogenesis of BHV-1 infection in neonatal and weanling calves by characterizing the competence of the immune system. Although the immune response was similar in both age groups, the neonates responded slower and failed to eliminate the virus. A lack of priming, rather than an inability to respond correctly appears to partially account for the increased susceptibility of neonates to BHV-1.

PROJECT NO. 2.4-22, FINAL REPORT, MARCH 1990

Summary

A variety of mechanisms have been suggested to account for the inability of neonatal animals to adequately respond to virus infections. The number or type of cells which respond to the virus, or the ability of these cells to secrete substances which can control or eliminate the virus can be examined in the laboratory. The overall objective of this project was to compare bovine herpesvirus-1 (BHV-1) infection in neonatal and weanling cattle, by characterizing the type of cellular response in the blood and lymphoid tissue, and to measure the ability of different white blood cells to secrete substances which might control the outcome of the virus infection.

Two studies were done. The pharyngeal tonsil is an important lymphoid tissue, located in the back of the throat. This tissue is affected by BHV-1 infection, but there have been few studies on the normal structure in cattle. Therefore, the first study was required to provide background information on the normal structure of the pharyngeal tonsil in cattle of various ages.

The second study consisted of 2 different experimental infection trials. In the first trial, neonatal or weanling calves were given BHV-1 by nasal spray, and a variety of tissues and blood samples were collected at 24 hour intervals for up to 6 days in the neonates and 8 days in the weanlings. Neonatal calves generally die at or around 7 days post-infection. The second trial was similar, but up to 6 infected and 6 control calves of each age group were sampled at a single time point of 4.5 days after infection with BHV-1.

The clinical disease and lesions produced by the virus were similar in neonatal and weanling cattle, but neonatal calves do not recover from the infection by 6 days post-infection. Examination of normal lymphoid tissue, particularly the pharyngeal tonsil indicated that the neonates have a reduced number of cells, lack evidence of previous activity (priming) and that there is a difference in the predominant type of white blood cell. Although the tissue location and the type of white cell responding to the infection were similar in both age groups, the neonatal calves did not respond as rapidly or as intensely as the weanlings. Furthermore the lesions in the neonatal calves did not begin to repair by 6 days post-infection, but repair was present in the weanlings at 5 or 8 days post-infection. Based on the immunocytochemistry and virus isolation, the weanling calves but not the neonates, had eliminated the virus by 8 days post-infection. Similarities were found in the ability of the neonates and weanlings to produce anti-viral and inflammatory mediators including serum interferon and prostaglandin, respectively. Serum cortisol was elevated in the neonates but reduced in the weanlings, and neutrophil chemotaxis was unaffected in the neonates but reduced in the weanlings post-infection.

Overall, the type of cells and number of cells responding to the infection appeared similar in the neonates and weanlings. However, at a similar time points post-infection, the neonates had a less intense response, and the cells were slower to begin responding to BHV-1 infection. A prominent

difference in the normal structure of lymphoid tissue in neonates is the lack of germinal centers, which indicate priming and a functional readiness of the tissue. In the weanling calves the virus was found to be present in the germinal centers several days before the virus was eliminated and the lesions caused by the virus were repairing. In contrast, the neonatal calves did not eliminate the virus and the lesions were not repaired at the last time point of 6 days. The slowness of the immune response (due to lack of priming), and failure to eliminate the virus appear to be important determinants controlling BHV-1 infection in cattle.

Previous studies on BHV-1 have not attempted to correlate the pathologic alterations with the immunologic alterations. The multidisciplinary approach to this study has extended our knowledge on the basic anatomy and development of lymphoid tissue in the bovine. The results of this study indicate that the immune system of neonates is probably as competent as that in weanlings for responding to BHV-1 infections. The main difference appears to be the lack of priming and the slowness of the immune system in eliminating the virus in the neonates. This information suggests that stimulation of the priming mechanism in the lymphoid tissue in calves at birth could be used to reduce the severity of viral and bacterial infections which commonly kill calves under 10 days of age, or to reduce the cost of treating these conditions. Immunocytochemistry with monoclonal antibodies was used to label the virus and to identify the different types of white blood cell in the tissues. The test for BHV-1 was confirmed as a reliable, specific and sensitive method to locate the virus for diagnostic purposes.

(b) TECHNICAL REPORT

Abstract

Bovine herpesvirus-1 (BHV-1) causes several economically important diseases of cattle, including infectious bovine rhinotracheitis and death of neonatal calves. The reason for the fatal infection in neonates is thought to be due to the inability of the immune cells to properly modulate the infection. However, the exact nature of the defect and the response of solid lymphoid tissue to BHV-1 infection has not been characterized.

The objective of this research was to compare the pathogenesis of BHV-1 infection in neonatal and weanling calves as a means of evaluating immunocompetence of the lymphoid tissue in the upper and lower respiratory tract. A study of the normal anatomy of the pharyngeal tonsil, primarily using specimens from the abattoir and other experimental studies, was undertaken to provide background information needed to properly interprete changes in this structure. Neonatal or weanling calves infected with a 5 minute intranasal aerosol of strain 108 of BHV-1 were examined at 24 hour intervals for up to 6 days (neonates) or 8 days (weanlings). The second portion of the experimental trials examined up to 6 infected, and 6 control neonatal and weanling calves at a single time point of 4.5 days post-infection. Tissues and blood samples were collected for histopathology, immunocytochemistry and functional assays and were used to characterize the competence of the immune system.

The clinical response and type of lesions produced by the virus were similar in both age groups. However, the inflammatory response was slower in the neonates. In the neonates, the damage subsequent to viral infection was less severe in the lymphoid tissue of the upper respiratory tract (particularly the pharyngeal tonsil), but more severe in the lung., compared to the weanlings. The neonates also failed to eliminate the virus from the pharyngeal tonsil and lung. The neonates were as capable as the weanlings in secreting interferon and prostaglandin. Therefore, it appears that once activated, the competence of the immune response (type of cell and secretions from cells) is similar in the neonates and weanlings. However, the lag in the inflammatory response, and subsequent failure to clear the virus from affected tissues contributes to the increased susceptibility of neonatal calves to bovine herpesvirus infections.

Background and Objectives

Bovine herpesvirus-1 (BHV-1), is an economically important virus of cattle which causes a self-limiting respiratory tract disease in older calves and adults (infectious bovine rhinotracheitis (IBR), "red-nose"), predisposes to fatal bacterial pneumonia, causes abortion and reproductive failure, and death of neonatal calves. Compared to older calves, neonatal calves are unable to control the virus infection. The inability of white blood cells to

properly respond to, or modulate the infection is the likely cause, but the exact mechanism is unknown. The functional response of the white blood cells, and a comparison of the lymphoid tissue responses in neonatal versus older animals has been characterized in only a few viral infections. In previous studies of BHV-1, the competence of the immune system has focused on older animals, particularly the events occuring in the peripheral blood and not lymphoid tissues. The availablity of monoclonal antibodies to readily identify the virus in tissue sections, and to separately identify the type of leukocyte responding to the infection provides a useful technique to diagnose this virus infection and to better characterize the immune response as it occurs within tissues of the animals.

The initial infection trial (sequential weanling study) with BHV-1 indicated that the pharyngeal tonsil was affected by the virus. However, it quickly became apparent that information on the normal anatomy and ontogeny of this tissue was not available. Therefore, a study on the ontogeny and immunophenotyping of the pharyngeal tonsil in cattle, as a function of age, was done. The results of this anatomical study were required to properly interprete the results of the pathology and the leukocyte phenotyping on the pharyngeal tonsils from the BHV-1 studies.

The overall purpose of this study was to characterize the pathogenesis and pathologic sequelae of BHV-1 infection in neonatal versus weanling calves, emphasizing the effect of the virus on the immune system of the upper and lower respiratory tract. The possible role of the pharyngeal tonsil in the pathogenesis of BHV-1 infection was emphasized in examination of the upper respiratory tract. Affected tissues were examined using histopathology, electron microscopy, and immunocytochemistry as a means to evaluate the effectiveness of the immune system, and to correlate the pathologic findings with the immunologic findings.

Experimental Methods

SECTION 1 Anatomy, ontogeny and immunophenotyping of the pharyngeal tonsil in cattle

Samples of pharyngeal tonsils were collected from 97 cattle at the abattoir or from experimental studies. Fetal, neonatal, weanling, yearling, adult and aged cattle were examined. Histology, scanning electron microscopy, and transmission electron microscopy were used to evaluate the development of this tonsil in cattle as a function of age. Semi-quantitative evaluation of tissue section stained with an immunoperoxidase method was used to identify the location and type of white cells present within the epithelium and submucosa at different ages.

SECTION 2 Immunopathogenesis of BHV-1 in neonatal versus weanling calves

Neonatal Holstein and Hereford-cross calves were obtained from the University of Saskatchewan herds at birth, and fed colostrum free of antibodies to BHV-1. The neonates were infected at 1.5-2.5 days of age. Weanling calves (5-months-old) were obtained from 2 Saskatchewan beef herds with no history of BHV-1 infection. All calves were infected with a 5 minute intranasal aerosol of BHV-1 (strain 108), and examined daily for evidence of clinical disease.

In the first experiment (sequential study), tissues were collected from neonatal calves (n = 1) at 1, 2, 3, 4, 5, and 6 days post-infection. Tissues from control calves were collected at 2 and 6 days post-infection. Tissues from weanling calves (n = 3) were collected at 1, 2, 3, 4, 5, and 8 days post-infection. Tissues from control calves were collected at 9 days post-infection. To provide sufficient data for statistical evaluation, a second experimental study (4.5 day study) was done to examine 5-6 calves at a single time point of 4.5 days post-infection.

The following samples were collected from the calves:

1) Peripheral blood for routine hematology, serum for virus neutralization assay, serum cortisol assay, serum interferon assay, and isolation of white blood cells for functional assays.
2) Tissues from the upper and lower respiratory tract for routine histopathology.
3) Pharyngeal tonsil for scanning and transmission electron microscopy.
4) Tissues from the upper respiratory tract for isolation of white cells for functional assays.
5) Tissues from the upper and lower respiratory tract were frozen for immunocytochemistry for identification of virus and phenotype of leukocytes responding to the infection.
6) Tissues for virus re-isolation.
7) Tissues from the upper and lower respiratory tract for bacteriology and mycoplasmology (lung).

For each group of calves, the following endpoints were measured:

1) Clinical response to infection
   Hematology pre-and post-infection
   Serum neutralization titer pre-and post-infection

2) Bacteriology (lung and tonsils)
   Mycoplasma (lung)

3) Virus re-isolation from tissues

4) Immunocytochemistry of tissues
   virus
   leukocyte phenotype
   double label for virus and leukocyte phenotype

5) Pathology
   - gross
   - histopathology
   - transmission and scanning electron microscopy (pharyngeal tonsil)
   - morphometry

6) Immune function
   - neutrophil chemotaxis
   - interferon
   - serum prostaglandin
   - serum cortisol
   - interleukin-1
   - interleukin-2

Previously described techniques for the immune function assays, and a modification of an immunoperoxidase staining procedure were used.

Where appropriate, the Student's t test was used for statistical evaluation of the results.

Results and Discussion

SECTION 1 Anatomy, ontogeny and immunophenotyping of the pharyngeal tonsil in cattle

The following list includes the important findings:

1. The pharyngeal tonsil is present as early as 95 days of gestation.
2. The furrows and rugae increase in size with increasing age.
3. Surface specilization includes invaginations which deepen with age.
4. Patches of non-ciliated epithelium (M cells) are scattered over the surface of the rugae and at the edge of the surface invaginations.
5. B cells are the earliest cell population in the submucosa, and predominate in fetal pharyngeal tonsils.
6. T cells predominate in the submucosa of postnatal cattle.
7. Lymphoid follicles are poorly developed at birth and germinal centers do not develop until calves are 2-4 weeks of age.
8. Intraepithelial leukocytes appear late in gestation and cells positive for the monocyte/granulocyte marker predominate in late gestation and neonatal cattle. The BoCD8 phenotype predominates in older calves.
9. The lymphoid tissue involutes in cattle over 7 years of age.

Age-related structural differences, and differences in the immunophentype of the submucosa and intraepithelial leukocytes which could potentially be a factor in the pathogenesis of disease were identified in this study.

SECTION 2 Immunopathogenesis of BHV-1 in neonatal versus weanling calves

1) Clinical response to BHV-1 infection

Both neonatal and weanling calves responded to BHV-1 infection by developing a statistically significant increase in body temperature beginning 2 days post-infection (Figure 1). At 2-3 days post infection, the calves became hyporexic, mildly depressed, and developed rhinorrhea or a mucopurulent exudate, a cough and increased lung sounds. At 8 days post-infection, the weanling calves were clinically recovered from the infection, but the neonatal calf at 6 days post-infection was still clinically ill. These findings are consistent with other reports of the clinical signs of BHV-1 infection in cattle.

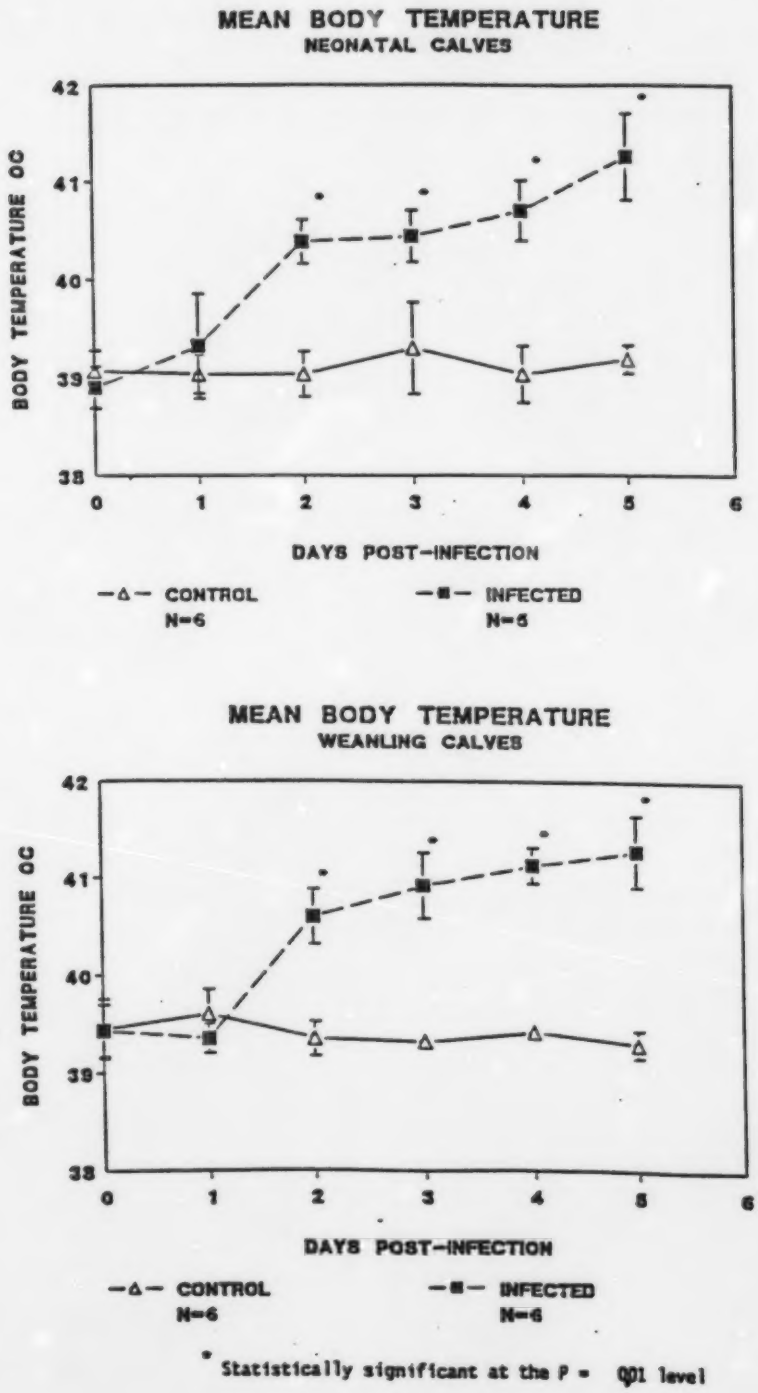

FIGURE 1. Mean ± standard deviation of body temperature of calves infected with bovine herpesvirus-1 (4.5 day post-infection study).

Hematology

In the majority of the neonatal calves, there was a large increase in the number of band neutrophils, and toxic change in the neutrophils. In the weanling calves there was a statistically significant decrease in the number of segmented neutrophils. In both age groups there was a trend towards an increase in circulating monocytes. These results confirm the increased severity of the infection in the neonates (presence of toxic neutrophils). The altered neutrophil populations in the neonates and weanlings indicates an increased tissue demand in response to BHV-1 infection. The difference in the neutrophil dynamics is a result of the difference in the dynamics of neutrophils in normal neonatal calves.

Serum neutralization titer pre-and post-infection

One weanling calf used at the 4 day post-infection time point (sequential study) was found to have antibodies to the virus. This animal did not develop clinical signs or lesions of BHV-1 infection.

2) Bacteriology (lung and tonsils)

Pasteurella haemolytica, Actinomyces pyogenes, and Listeria monocytogenes were infrequently isolated from the tissues, indicating no complicating or secondary bacterial infections.

Mycoplasma (lung)

Mycoplasma sp. was not isolated from the tissues.

3) Virus re-isolation from tissues

BHV-1 was re-isolated in nearly all cases from the lung and pharyngeal tonsil, less frequently from the palatine tonsil and rarely from lymph nodes or visceral organs. Virus was not re-isolated from the lung or pharyngeal tonsil of the weanling calves examined at 8 days post-infection, indicating that the virus had been cleared from these sites.

4) Immunocytochemistry of tissues

- identification of BHV-1

Identification of the virus in tissues using the immunoperoxidase technique, correlated well with the results of the virus re-isolation and the stage of the lesion. In only a few cases, a small amount of virus was found by virus isolation and not found on immunoperoxidase staining. Virus could not be found in the tonsils and lung of the weanling calves at 8 days post-infection, but was still found in the lung the neonate at 6 days post-infection. Notably, virus was found deep in the germinal centers of the tonsils in weanling calves, in a manner suggesting antigen presentation and

processing. Neonatal calves do not have germinal centers and the virus was present only in the epithelium. The ability of the virus to move to germinal centers is likely important in the elimination of the virus from the tissues of these older calves and not the neonates.

- leukocyte phenotype

Preliminary results indicate that the virus induces an increase in a subset of the T cell population (BoCD8 - cytotoxic/suppressor cells) in both age groups. The increase in the number of cells positive for the MHC class II marker appears to be greater in the weanlings. The neonates, but not the weanlings have a slight increase in the number of B cells.

- double label for virus and leukocyte phenotype together

A successful double label technique has only recently been developed. The virus does not appear to directly infect leukocytes. Further evaluation of the tissues will be necessary to confirm this result.

5) Pathology

- gross

In both age groups BHV-1 infection causes multifocal to coalescing necosis with fibrin exudation, in the upper respiratory tract (nasal cavity, pharyngeal tonsil, epiglottis and upper trachea). The lung lesions consisted of atelectasis and pneumonia. The lymph nodes draining the respiratory tract were enlarged and edematous. These findings are consistent with other reports of BHV-1 infection. However, few reports have documented lesions in the tonsils, particulary the pharyngeal tonsils.

- histopathology

Erosions and ulcers of the respiratory epithelium, with intranuclear inclusion bodies and leukocyte exudation were the characteristic lesions. The lesions were being repaired in the weanling calves by 8 days post-infection, whereas the lesions were still active in the neonatal calf at the last time point. The lesions in the upper respiratory tract were more severe in the weanlings, and the lesions in the lung were more severe in the neonates. A comparison of the lesions at identical time points indicated that the character of the inflammatory response was similar in both age groups, but the inflammatory response was slower and generally less extensive in the neonatal calves. The enlargement of the lymph nodes appeared to be the result of expansion of cell populations in all areas of the tissue. Thymic atrophy was present in the neonatal and weanling calves at the last 2 time points examined, and is consistent with stress.

- transmission and scanning electron microscopy (pharyngeal tonsil)

The virus was found in both types of epithelial cells, and the amount of virus replicating in individual cells was similar in both neonates and weanlings. The loss of cilia from the cells was one of the earliest changes noted. The phagocytic cells were as active in the necrotic areas in the tissues from the neonates, as from the weanlings. This suggests that these cells function in the neonates at the same level as the weanlings. However, the function of the microbial killing pathways may still be defective, but could not be evaluated.

- morphometry

The thymic atrophy is the result of loss of cortical lymphocytes. Preliminary results indicate that the enlargement of the lymph nodes is the result of expansion in the medulla and cortical region. Further analysis is underway.

6) Immune function

- neutrophil chemotaxis

In unaffected calves, there is no difference in the chemotactic response of the neonates and weanling calves. Once infected with BHV-1, there is a statistically significant decrease in the chemotactic ability of the neutrophils in the weanlings but not the neonates (Figure 2). These results indicate that the chemotactic ability of neutrophils in neonates is unaffected by virus. The decrease in chemotactic ability of neutrophils in the weanlings has been previously reported, and is thought to be involved in the increased susceptibility of these calves to secondary fatal bacterial pneumonias.

- interferon

Serum interferon levels were statistically increased after infection with BHV-1 in both neonates and weanlings (Figure 3). This indicates that one of the main anti-viral secretions is produced in neonates and is likely not the reason for the fatal infection in the neonates.

- serum prostaglandin $E_{2\alpha}$

There was no difference in the prostaglandin production in the neonates and weanlings (Figure 4). Therefore, production of this factor is not affected by age, and the immunosuppressive activity or inflammatory activity of prostaglandin is likely not involved in the inability of neonatal calves to control BHV-1 infection.

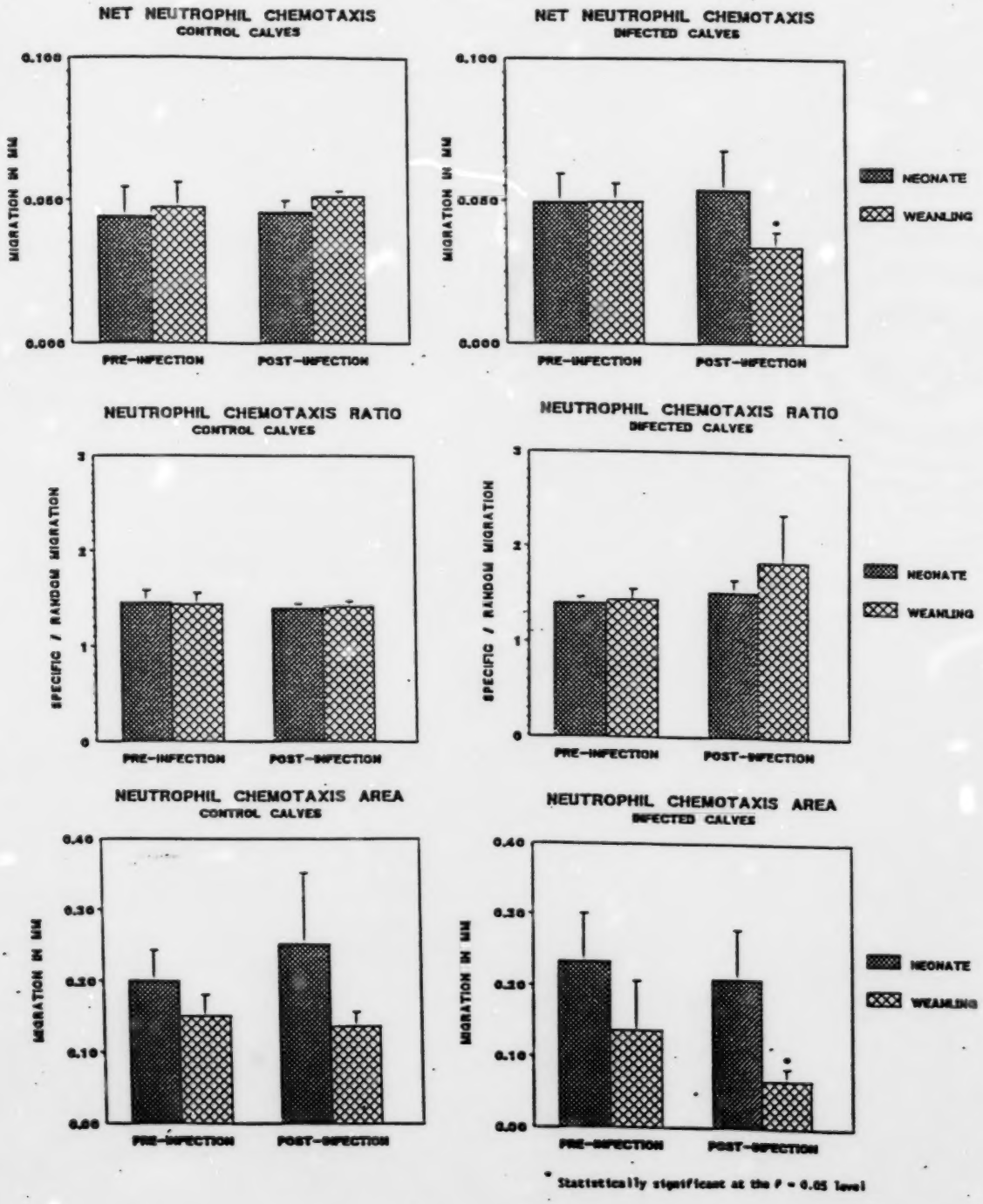

FIGURE 2. Neutrophil chemotaxis assay (mean ± standard deviation) for calves infected with bovine herpesvirus-1 (4.5 day post-infection study).

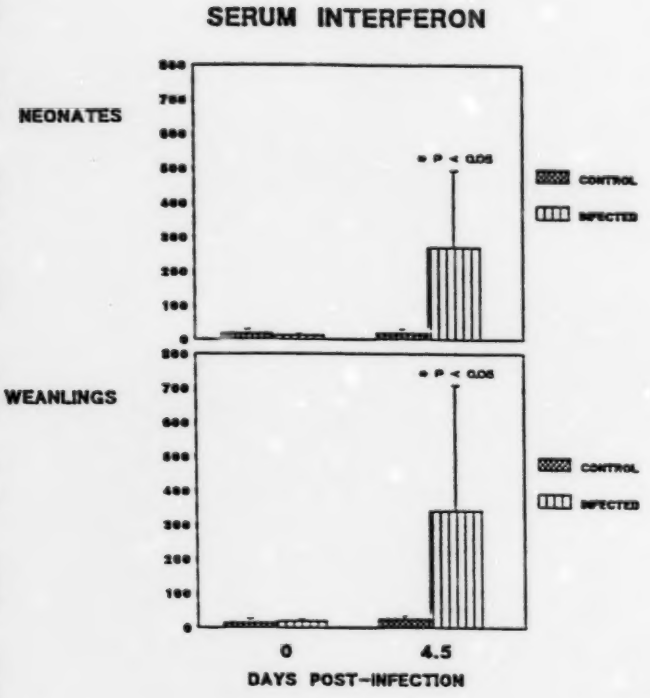

FIGURE 3. Serum interferon for calves infected with BHV-1.

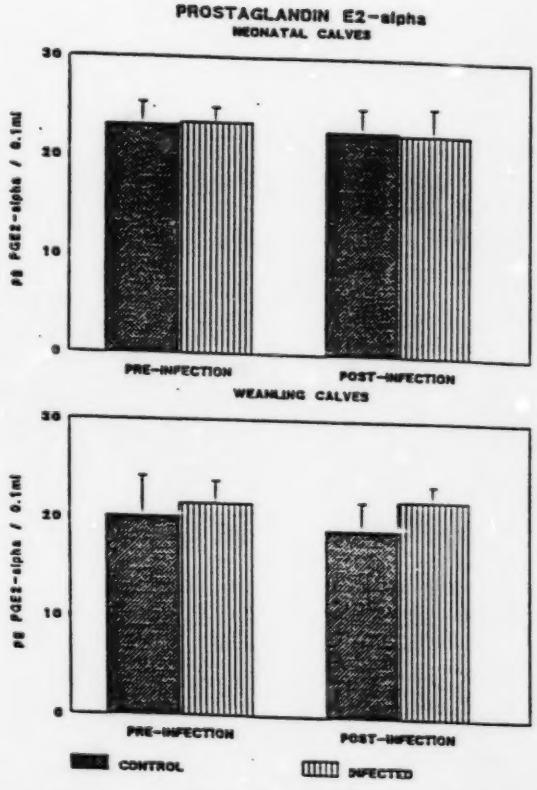

FIGURE 4. Serum prostaglandin for calves infected with BHV-1.

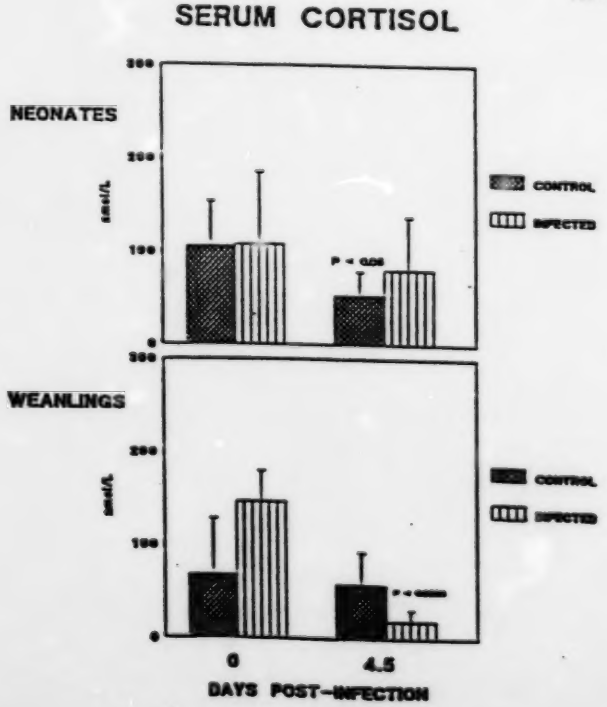

FIGURE 5. Serum cortisol for calves infected with BHV-1.

- serum cortisol

In neonatal calves cortisol levels are elevated at birth. The normal decrease in cortisol levels which occurs within the first week of life was not present in the neonates infected with BHV-1 (Figure 5). This is consistent with the stress that disease places on young calves. In the weanling calves, the cortisol levels were variable and decrease with BHV-1 infection. These results are not consistent with previous studies which indicated an increase in cortisol levels in weanling calves post-infection. The cause of this discrepancy is not known.

- interleukin-1 and interleukin-2

These assays have not been completed.

Conclusions

The following conclusions were made from this study:

SECTION 1 Anatomy, ontogeny and immunophenotyping of the pharyngeal tonsil in cattle

1. Although developed at birth, the lack of functional priming (lack of germinal centers) and difference in the number and phenotype of the available leukocytes may prevent neonates from adequately responding to aerogenously acquired microbial infections.

2. The lack of well-developed lymphoid follicles and complete lack of germinal centers in the lymphoid tissue of neonates is likely an important morphological difference which affects the competence of the pharyngeal tonsil and how it responds to disease. Fewer intraepithelial leukocytes in the epithelium of the pharyngeal tonsil of the neonates may also be important in disease response.

3. The pharyngeal tonsil is situated in an ideal location to sample aerogenously delivered antigens and likely plays an important role in the early response to infections such as BHV-1.

4. The location, structure and early development support the pharyngeal tonsil and not the bronchus-associated lymphoid tissue as the most important discrete lymphoid stucture in the respiratory tract.

SECTION 2 Immunopathogenesis of BHV-1 in neonatal versus weanling calves

5. The clinical signs in response to the virus are similar in both age groups. The weanlings begin to recover by 5-8 days post-infection, and the neonates are still clinically ill at 6 days post-infection.

6. In both age groups the response of the neutrophils indicates a tissue demand for neutrophils at the sites of infection.

7. No significant quantities of bacteria, nor mycoplasma were isolated from the lungs or tonsils of the control or infected calves.

8. Virus re-isolation from tissues generally correlated with the findings by immunocytochemistry and the histopathology. In a few cases (particularly palatine tonsil), immunocytochemistry was negative but virus was isolated and lesions were found. This is likely a result of sampling error.

9. The tissues affected by the virus are similar in neonates and weanlings. The lesion in the pharyngeal and palatine tonsil is more severe in the weanlings and the lesion in the lung is more severe in the neonates.

10. The character of the inflammatory response is similar in both age groups, but the magnitude of the response is less in neonates.

11. The inflammatory response develops slower in the neonates than in the weanlings.

12. The weanlings repair the lesions in the upper and lower respiratory tract and clear the virus from these tissues by 8 days post-infection. Neonates do not show evidence of repair in the upper respiratory tract, limited type II pneumocyte hyperplasia in the lung, and do not clear the virus from tissues by 6 days post-infection.

13. The earliest lesion in the pharyngeal tonsil is loss of cilia and goblet cells. Both the ciliated and non-ciliated cells are affected by the virus.

14. By electron microscopy, phagocytic cells of the neonates are as active as the phagocytic cells of the weanlings.

15. Neutrophil chemotactic ability of the neonates is unaffected by viral infection, whereas the chemotactic ability of the weanlings is decreased by viral infection.

16. Prostaglandin $E_{2\alpha}$ levels are similar in the neonates and weanlings of both control and infected calves, and the levels do not change with BHV-1 infection.

17. Serum interferon levels are similarly increased in neonatal and weanling calves after BHV-1 infection.

The inability of neonatal calves to modulate BHV-1 infection does not appear to be the result of a specific defect in any one cell type or the secretory ability of immune cells which respond to the presence of the virus. Rather, the lack of priming and the slow initiation of the inflammatory response, and a failure to eliminate the virus from the tissues of the upper and lower respiratory tract appear to be the main factors involved in the inadequate immune response.

Impact on Agriculture

Previously, most studies on BHV-1 were based on the traditional approach of examining the disease using techniques in pathology, immunology or virology. This study integrated these three disciplines, to provide a correlation of the pathologic alterations with the immunologic alterations.

This study has extended the scientific communities knowledge in understanding the basic anatomy and development of the lymphoid tissue, and in the pathogenesis of herpesvirus infections in young calves. Ultimately, development of a reliable method to prevent and control diseases such as BHV-1 requires understanding how the animals immune system responds to the infection. The results of this study have indicated that the immune system of neonates is probably as competent as that in weanlings, but the immune response is not initiated as fast in the neonates. This suggests that a method should be developed to stimulate the priming of the lymphoid tissue in calves at birth. This could be used to help control viral, and possibly bacterial infections which commonly kill calves under 10 days of age, and to reduce the amount of expensive drugs used to treat these conditions.

Immunocytochemistry with monoclonal antibodies was used to identify BHV-1, and to identify the different types of white blood cell in the tissues. The test for BHV-1 was confirmed as a reliable, specific and sensitive method to locate the virus for diagnostic purposes.

(c) LIST OF PAPERS PREPARED AND TALKS GIVEN

Manuscripts submitted

1. Schuh J.C.L., Oliphant L.W. and Doige C.E. Fetal and postnatal development of the pharyngeal tonsil in cattle: Surface topography, histology, ultrastructure and immunophenotyping. Submitted to "American Journal of Anatomy".

Manuscripts in preparation

2. Schuh J.C.L., Bielefeldt Ohmann H., Doige C.E. and Babiuk L.A. Pharyngeal tonsil lesions in neonatal versus weanling calves infected with bovine herpesvirus-1. To be submitted to "Veterinary Pathology".

3. Schuh J.C.L., Bilelefeldt Ohmann H., Babiuk L.A. and Doige C.E. Immunohistologic and immunofunctional studies of pulmonary and lymphoid tissues from neonatal and weanling calves acutely infected with bovine herpesvirus-1. To be submitted to "Canadian Journal of Veterinary Research".

Presentations

1. Cattle herpes project underway. Cattlemen magazine, October 1988, p. 42.

2. Schuh J.C.L., Oliphant L.W. and Doige C.E. Anatomy, ontogeny and immunophenotyping of the pharyngeal tonsil (adenoid) in cattle. Published in Allergologie 12:310, 1989. Presented at: The International Symposium on Mucosal Immunity of the Respiratory Tract, Bad Lippspringe, FRG, July 1989.

3. Schuh J.C.L., Bielefeldt Ohmann H., Babiuk L.A., and Doige C.E. Pathology of the pharyngeal tonsil in bovine herpesvirus-1 infected calves. Published in 1989 conference proceedings. Presented at: The 40th Annual Meeting of the American College of Veterinary Pathologists, Baltimore, Maryland, October 1989.